

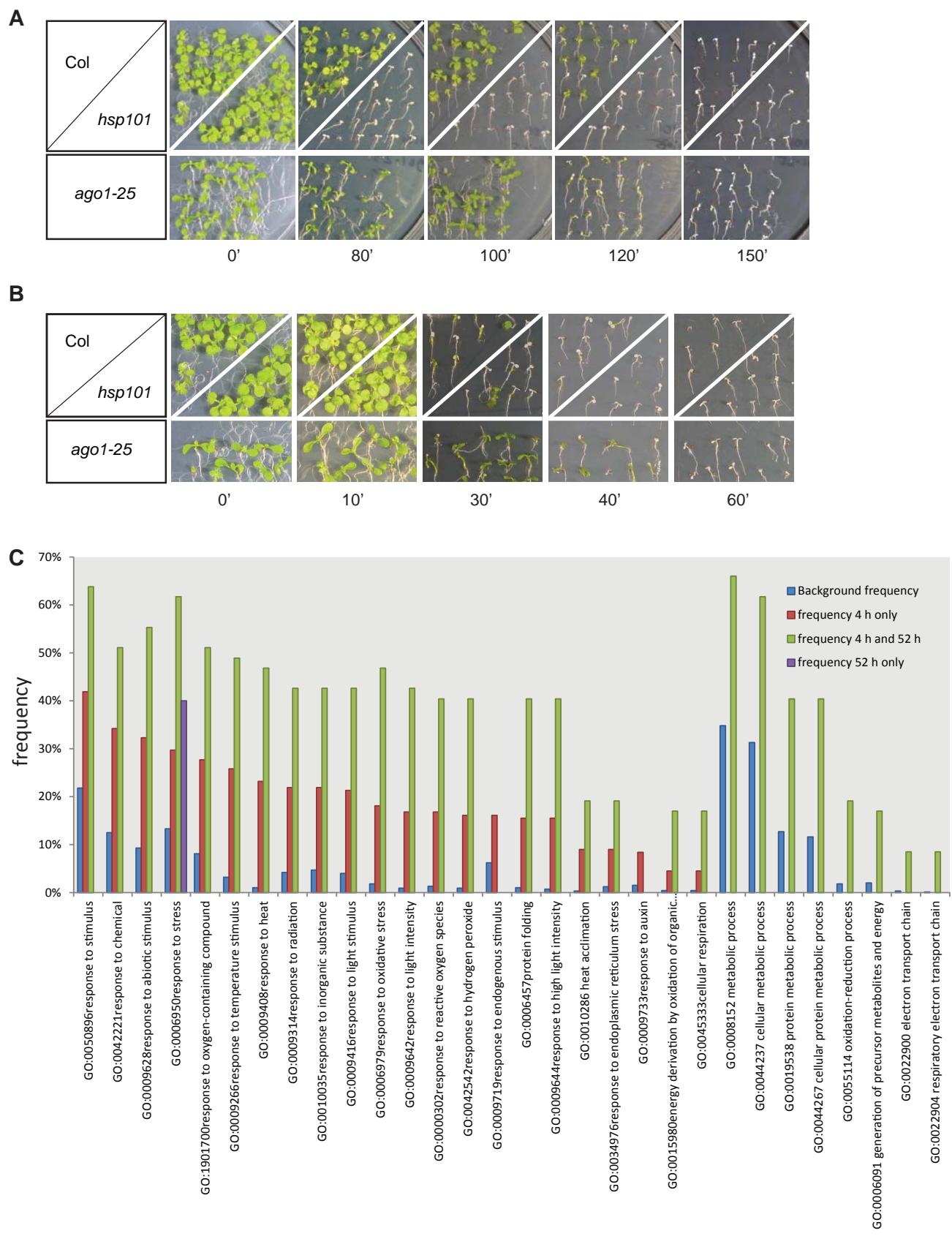
Supplemental Figure 1: Acquisition of thermotolerance and basal thermotolerance in *ago1-25* and GO term frequency analysis of ATH1 transcriptome profiling presented in Figure 3A.

(A) Acquisition of thermotolerance is largely unaffected in *ago1-25*. Acquisition of thermotolerance in Col-0, *hsp101*, and *ago1-25* was assayed by extending the second HS of the priming HS treatment for the indicated times (cf. Figure 1B). Photographs were taken 8 d after the HS.

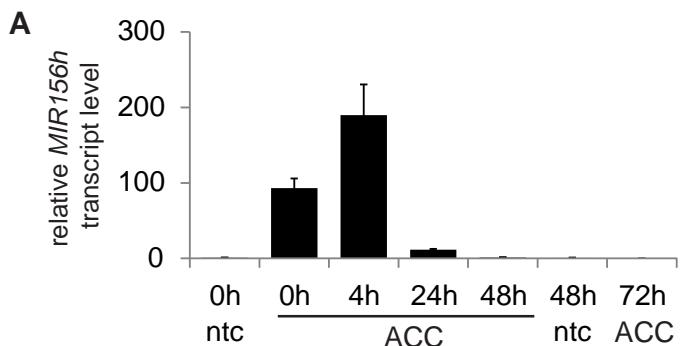
(B) Basal thermotolerance is unaffected in *ago1-25*. Basal thermotolerance in Col-0, *hsp101*, and *ago1-25* was assayed by incubating 3-day-old seedlings for the indicated times at 44°C (cf. Fig. 1A). Photographs were taken 8 d after the HS.

All plants of one treatment were grown on the same plate. The experiments were repeated at least 3 times with similar results.

(C) Significant enrichment (<0.01) of GO terms in the three classes of upregulated genes from the transcriptome profiling (Figure 3A). The frequency of genes with a particular GO term in a class is shown as well as the frequency of genes with this particular GO term among all TAIR-annotated genes (background frequency). The “4 h only” and “4 h and 52 h” classes both have a high enrichment for stress- and HS-related terms. The analysis was performed using the AmiGO webtool.



Supplemental Figure 1



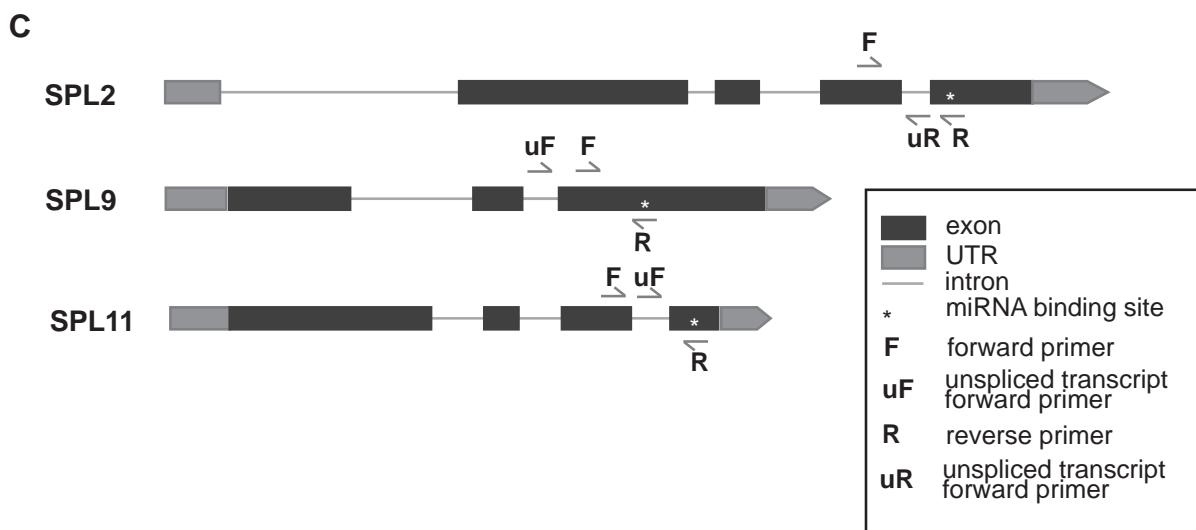
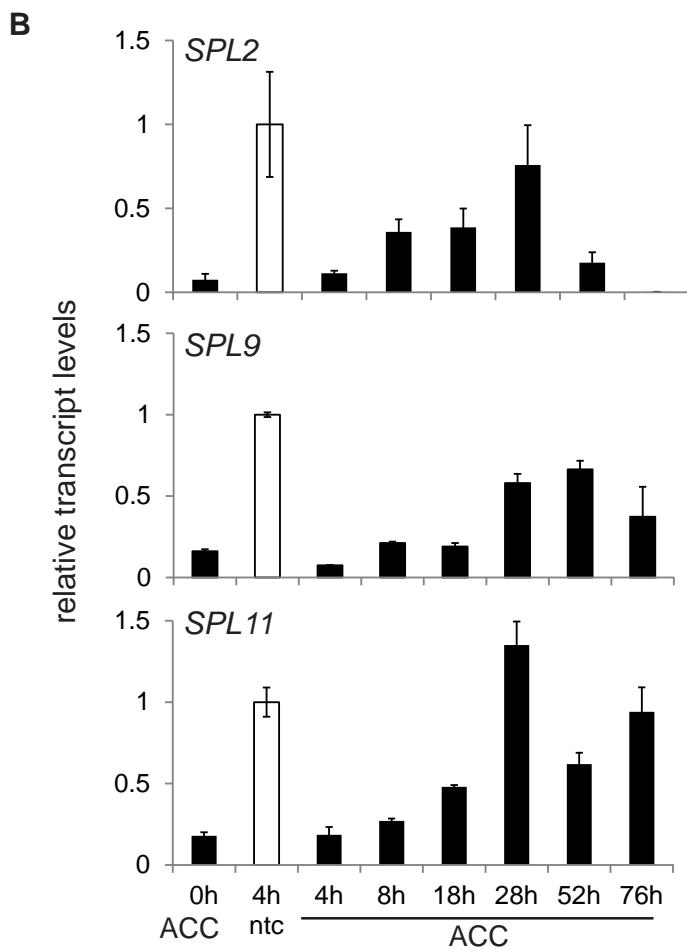
Supplemental Figure 2: *MIR156h* and *SPL2*, *9*, *11* expression levels (additional timepoints) and primer positions.

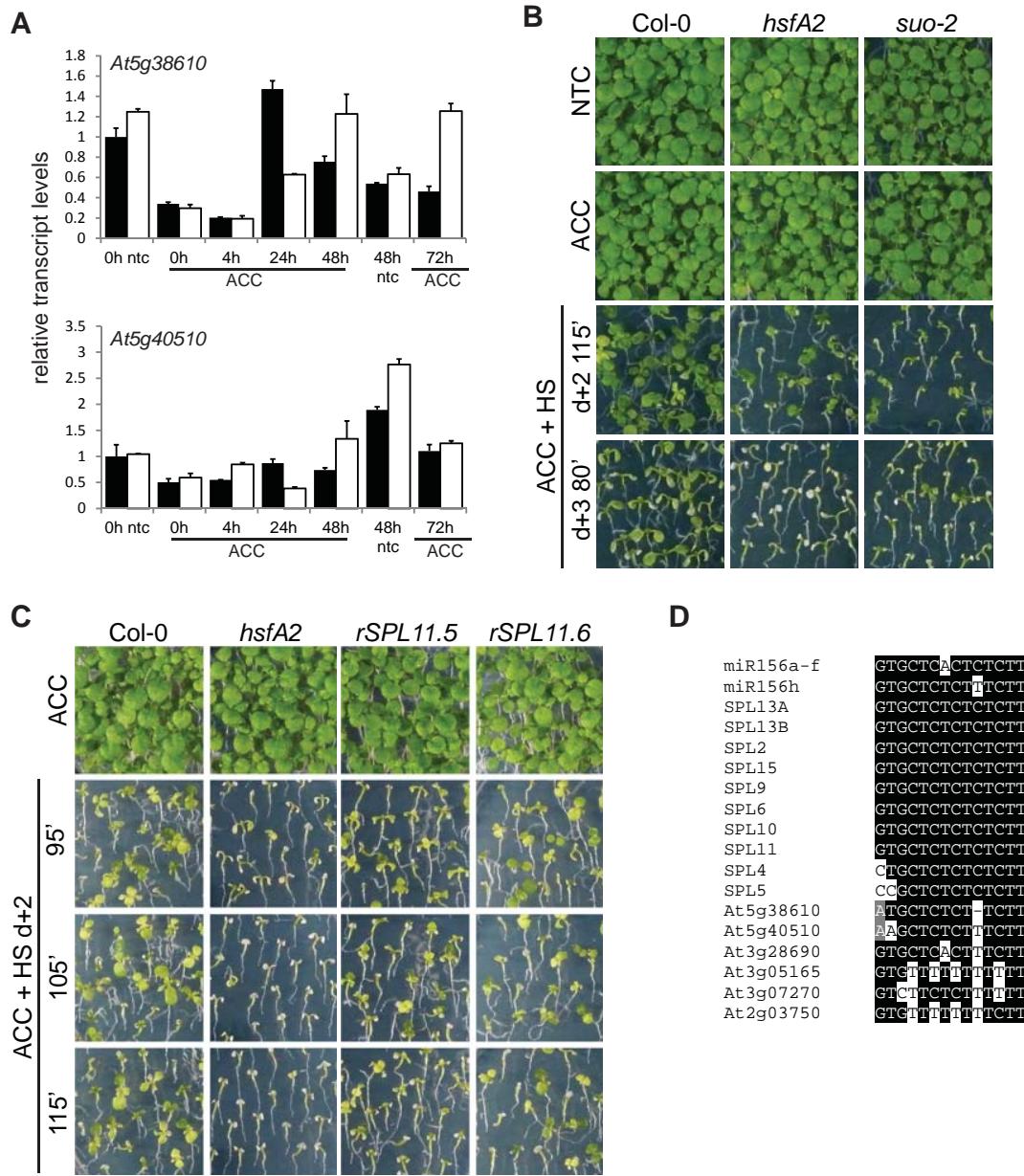
(A) Transcript levels of *MIR156h* matching the timepoints shown in Figure 5A.

(B) Transcript levels of *SPL2*, *SPL9*, *SPL11* matching the timepoints shown in Figure 4B.

Expression values were determined by qRT-PCR and normalized to *TUB6* and ntc. Error bars are SEM of three replicates.

(C) *SPL2*, *9*, *11* gene models and primer positions for detection of mature (spliced) and unspliced (nascent) transcripts. Mature transcripts were detected with primers F and R, unspliced transcripts were detected with primers uF and R (*SPL2*: F and uR).





Supplemental Figure 3: Expression profile of new *miR156h* target genes after a priming HS and requirement of SUO and the miRNA-binding site of *SPL11* for maintenance of acquired thermotolerance.

(A) Transcript levels of potential *miR156h* target genes after a priming HS in Col-0 (black bars) and *ago1-25* (white bars) determined by qRT-PCR. Expression values were normalized to *TUB6* and Col-0 0h ntc. Error bars are SEM of three replicates. The experiment was repeated at least three times independently with one representative shown.

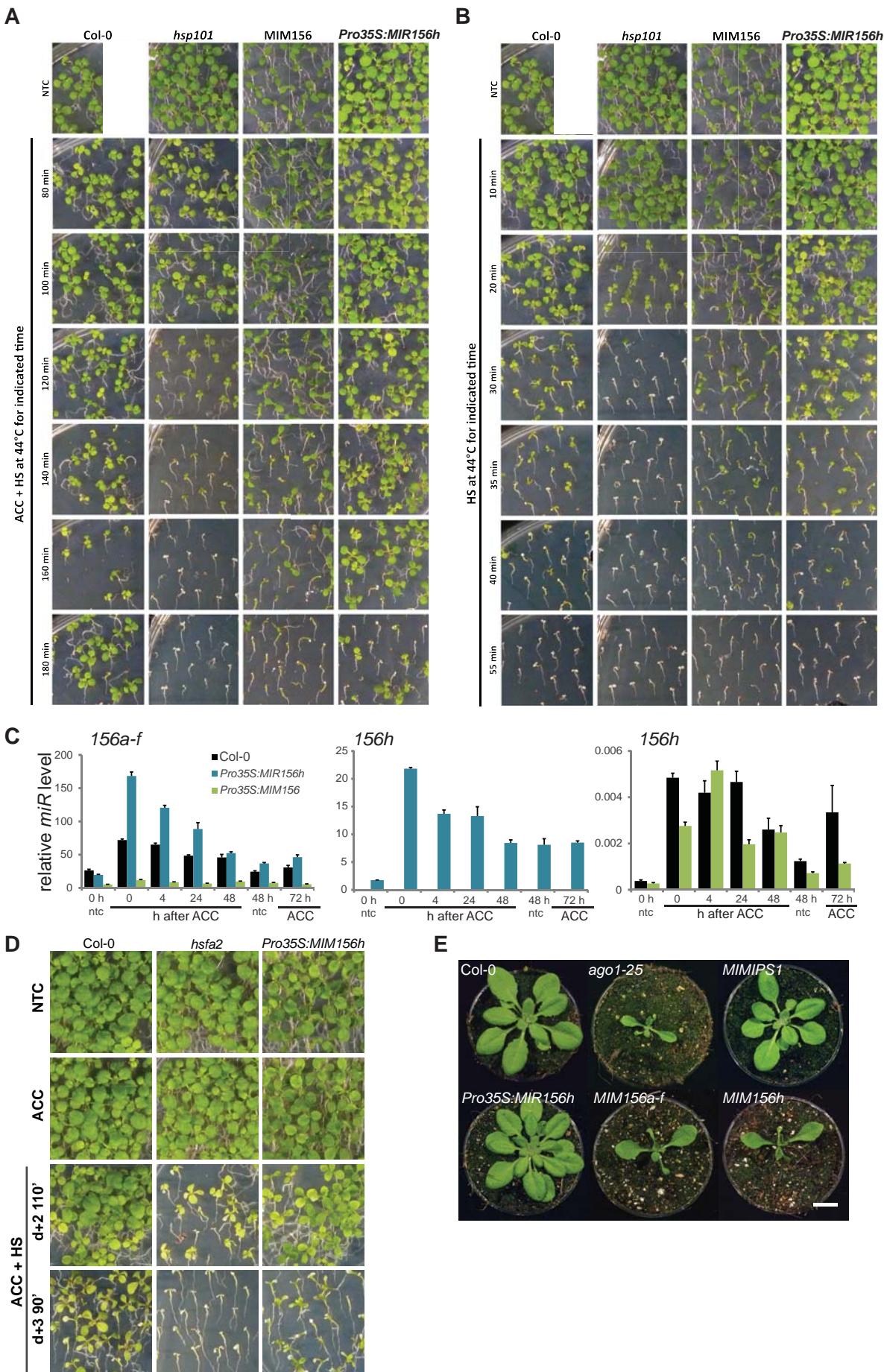
(B) *suo-2* mutant seedlings were assayed for the maintenance of acquired thermotolerance by applying a tester HS (HS) two (+2, 115 min) or three days (+3, 80 min) after a priming HS (ACC); *hsfA2* was included as a control. Photographs were taken 14 d after ACC. All plants of one treatment were grown on the same plate. The experiments were repeated at least three times with similar results.

(C) *SPL11::rSPL11* seedlings were assayed for the maintenance of acquired thermotolerance by applying a tester HS (HS) of varying length (95, 105, 115 min) two days after a priming HS (ACC). *rSPL11.5* and *rSPL11.6* represent independent transgenic lines. Photographs were taken 14d after ACC. All plants of one treatment were grown on the same plate. The experiments were repeated at least 3 times with two independent transgenic lines with similar results.

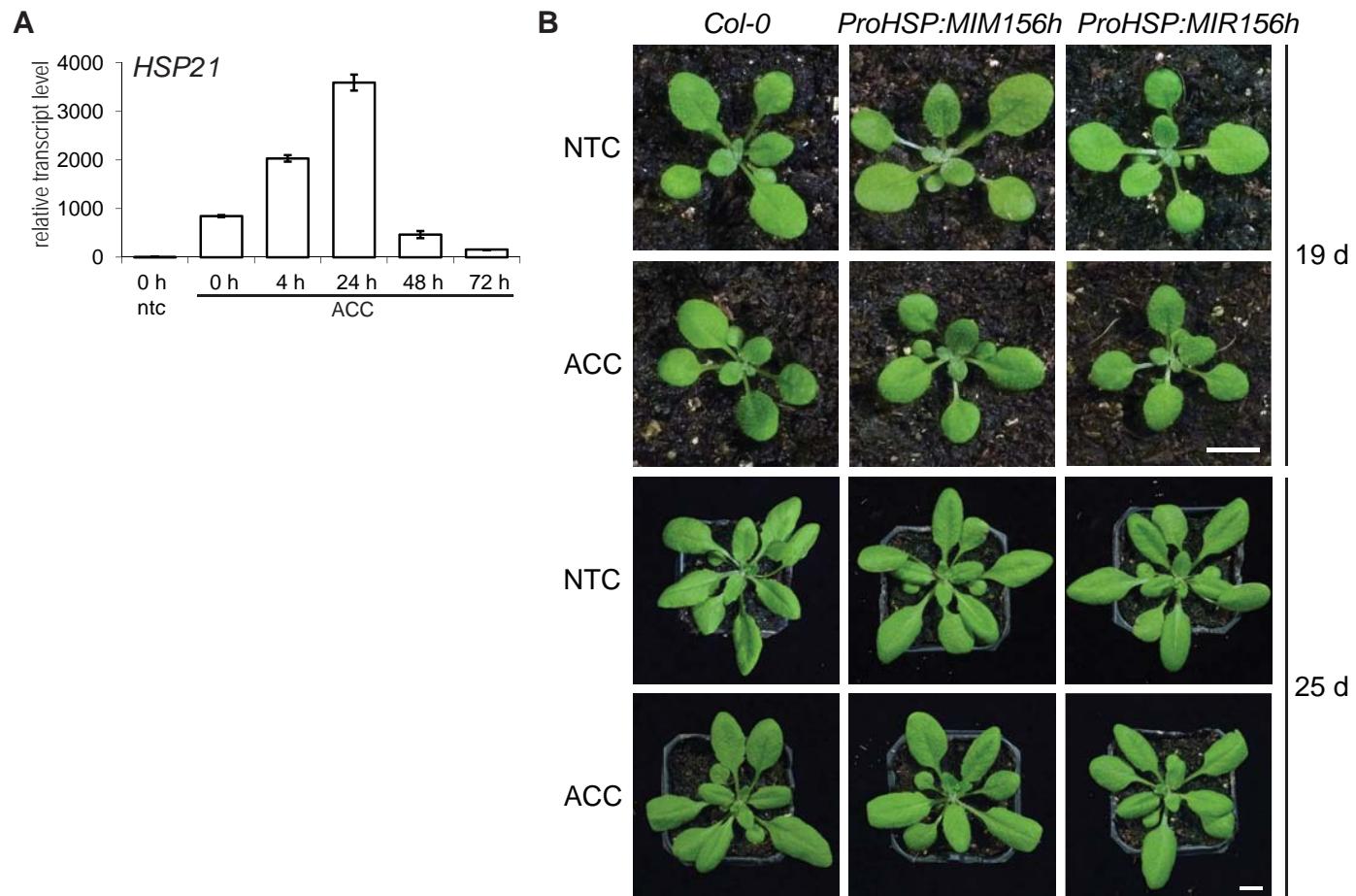
(D) Alignment of complementary *miR156a-f* and *miR156h* sequences with putative binding sites of target genes.

Supplemental Figure 4: Further characterization of *Pro35S:MIM156* and *Pro35S:MIR156h*.

- (A) Acquisition of thermotolerance is unaffected in *Pro35S:MIM156* and *Pro35S:MIR156h* plants. Acquisition of thermotolerance was assayed by extending the second HS of the priming HS treatment for the indicated times (cf. Figure 1B). Photographs were taken 8 d after the HS.
- (B) Basal thermotolerance is unaffected in *Pro35S:MIM156* and *Pro35S:MIR156h*. Basal thermotolerance was assayed by incubating 4-day-old seedlings for the indicated times at 44°C (cf. Figure 1A). Photographs were taken 8 d after the HS.
- (C) Mature miRNA levels of *miR156a-f* and *miR156h* in Col (black), *Pro35S:MIR156h* (blue) and *Pro35S:MIM156* (green) determined by qRT-PCR. Error bars are SEM of three replicates. The experiment was repeated at least twice independently and one representative is shown.
- (D) *Pro35S:MIM156h* plants have a defective maintenance of thermotolerance. *Pro35S:MIM156h* plants were assayed for the maintenance of acquired thermotolerance (cf. Fig. 1C) by applying a tester HS (HS) two (+2, 90 min) and three days (+3, 90 min) after a priming HS (ACC). Photographs were taken 14 d after ACC.
- (E) Effects of *Pro35S:MIM156a-f*, *Pro35S:MIM156h* and *Pro35S:MIR156h* on rosette phenotypes of 22-d-old plants grown in long days. Scale bar represents 1 cm. *MIMIPS1* (Franco-Zorilla et al., 2007) was included as a control.
All plants of one treatment were grown on the same plate. The results are representative for several experiments with homozygous lines derived from multiple independent transformants.



Supplemental Figure 4



Supplemental Figure 5: Further characterization of heat-inducible *miR156*-manipulating lines.

(A) Transcript levels of *HSP21* in *Col-0* after a priming HS as determined by qRT-PCR normalized to *TUB6* and 0h ntc.

(B) Nineteen- and 25 day-old rosette plants of the indicated genotypes treated with a priming HS (ACC) on day 4 or mock-treated (NTC). Scale bars represent 1 cm.

Supplemental Table 1: *MiR156a-f* and *miR156h* targeted genes predicted by at least two search tools and their gene description and expression.

| AGI | Name | Description | targeted by | changed at 4h in array | changed at 52h in array |
|-----------|--------|--|-------------|------------------------|-------------------------|
| At1g27360 | SPL11 | SPL11_squamosa promoter-like 11 | both | [REDACTED] | |
| At1g27370 | SPL10 | SPL10_squamosa promoter binding protein-like 10 | both | | |
| At1g53160 | SPL4 | SPL4_squamosa promoter binding protein-like 4 | both | | |
| At1g69170 | SPL6 | Squamosa promoter-binding protein-like (SBP domain) transcription factor family protein | both | | |
| At2g03750 | | P-loop containing nucleoside triphosphate hydrolases superfamily protein | h | | |
| At2g33810 | SPL3 | SPL3_squamosa promoter binding protein-like 3 | a-f | | |
| At2g42200 | SPL9 | AtSPL9_SPL9_squamosa promoter binding protein-like 9 | both | [REDACTED] | |
| At3g05165 | | Major facilitator superfamily protein | h | | |
| At3g07270 | | GTP cyclohydrolase I | h | | |
| At3g15270 | SPL5 | SPL5_squamosa promoter binding protein-like 5 | a-f | | |
| At3g28690 | | Protein kinase superfamily protein | both | | |
| At3g57920 | SPL15 | SPL15_squamosa promoter binding protein-like 15 | both | | |
| At5g38610 | | Plant invertase/pectin methylesterase inhibitor superfamily protein | h | [REDACTED] | |
| At5g40510 | | Sucrase/ferredoxin-like family protein | h | | [REDACTED] |
| At5g43270 | SPL2 | SPL2_squamosa promoter binding protein-like 2 | both | [REDACTED] | |
| At5g50570 | SPL13A | SPL13_SPL13A_Squamosa promoter-binding protein-like (SBP domain) transcription factor family protein | both | | |
| At5g50670 | SPL13B | SPL13_SPL13B_Squamosa promoter-binding protein-like (SBP domain) transcription factor family protein | both | | |

Supplemental Table 2: Average flowering time (days and total leaf number = TLN) and leaf initiation rate of *MIR156*-manipulating transgenic lines. Avg., average; sem, standard error of the mean.

| Line | | Flowering time (TLN) | | | Flowering Time (days) | | | Leaf Initiation Rate (leaves/day) | | n |
|------------------------------------|---------------|-------------------------|-----|-------|--------------------------|-----|-------|---|------|----|
| | | Avg. | sem | Range | Avg. | sem | Range | Avg. | sem | |
| Col-0 | | 16.0 | 0.7 | 13-22 | 29.2 | 0.6 | 27-34 | 0.50 | 0.02 | 13 |
| <i>hsfA2</i> | | 15.3 | 0.6 | 12-18 | 29.4 | 0.6 | 28-32 | 0.55 | 0.02 | 9 |
| <i>ago1-25</i> | | 11.8 | 1.3 | 6-16 | 37.3 | 1.6 | 32-41 | 0.33 | 0.04 | 8 |
| <i>Pro35S:</i> <i>MIM156a-f</i> | IB38. 2.1 | 4.8 | 0.9 | 1-8 | 25.2 | 3.0 | 23-32 | 0.15 | 0.03 | 9 |
| <i>Pro35S:</i> <i>MIM156a-f</i> | IB38.4 .4 | 6.6 | 0.6 | 4-10 | 28.0 | 1.2 | 23-32 | 0.21 | 0.02 | 10 |
| <i>Pro35S:</i> <i>MIR156h</i> | IB32. 7.1 | >66 | | | >46 | | | 1.63 | 0.08 | 6 |
| <i>Pro35S:</i> <i>MIR156h</i> | IB32. 14.7 | >66 | | | >46 | | | 1.62 | 0.04 | 10 |
| <i>Pro35S:</i> <i>MIMIPS1</i> | BP4. 5.3 | 20.1 | 0.4 | 19-22 | 33.3 | 0.4 | 32-34 | 0.64 | 0.02 | 9 |
| <i>Pro35S:</i> <i>MIMIPS1</i> | BP4. 12.6 | 20.8 | 0.7 | 18-25 | 31.8 | 0.5 | 30-35 | 0.65 | 0.02 | 10 |
| <i>Pro35S:</i> <i>MIM156h</i> | BP5. 1.1 | 13.8 | 0.6 | 11-16 | 29.6 | 0.6 | 28-32 | 0.49 | 0.02 | 9 |
| <i>Pro35S:</i> <i>MIM156h</i> | BP5. 4.6 | 7.8 | 0.5 | 6-10 | 28.0 | 0.7 | 23-32 | 0.28 | 0.02 | 10 |

Supplemental Table 3: Sequences of oligonucleotides used in this study.

| Gene | Name | Sequence | Application |
|-----------|-------------------|--|-------------------------------|
| | 429/miR831_F | CACTTGTGGTGTGTTACTCTGA | qRT-PCR pri-miRNA |
| | 430/miR831_R | GAGTGTCATAGAATCGCAAGAAGAGT | qRT-PCR pri-miRNA |
| | 433/miR2936_F | TTCAATCTCGTTGCCGCAGT | qRT-PCR pri-miRNA |
| | 434/miR2936_R | TCTTGTAGCTCTTGCGCCG | qRT-PCR pri-miRNA |
| | 435/miR156e_F | GTCACATGCGTAGAGTGTGAAAGG | qRT-PCR pri-miRNA |
| | 436/miR156e_R | CCATGTGTGCTCACTCTCTCTGT | qRT-PCR pri-miRNA |
| | 437/miR156h_F | TCCTCCCCAGAAGAAGATGAAAAA | qRT-PCR pri-miRNA |
| | 438/miR156h_R | GCTAATCCCAGGTTGCTCTCT | qRT-PCR pri-miRNA |
| | 461/miR391_F | AGAGATAGCGCCATCACCTCTT | qRT-PCR pri-miRNA |
| | 462/miR391_R | ACGTAGGAGAGATACCGTCACCA | qRT-PCR pri-miRNA |
| | 463/miR171b_F | TGCGGTTCAATCAAATAGTCGCTCTC | qRT-PCR pri-miRNA |
| | 464/miR171b_R | GGCACGGCTCAATCGAACACAC | qRT-PCR pri-miRNA |
| | atmiR156gStLRT | GTCGTATCCAGTGCAGGGTCCGAGGTATTGCACTGG ATACGACGTGCT | qRT-PCR |
| | atmiR156gTqmn_F | TCGACGCGACAGAACAGAGAGTGA | qRT-PCR |
| | miR156hStLpRT | GTCGTATCCAGTGCAGGGTCCGAGGTATTGCACTGG ATACGACGTGCTC | qRT-PCR |
| | miR156hTqMn_F | CGTCGACGTGACAGAACAGAAAGA | qRT-PCR |
| | miR391StLpRT | GTCGTATCCAGTGCAGGGTCCGAGGTATTGCACTGG ATACGACTGGCG | qRT-PCR |
| | miR391TqMn_F | GCACGTTGCAGGAGAGATAG | qRT-PCR |
| | miR831StLpRT | GTCGTATCCAGTGCAGGGTCCGAGGTATTGCACTGG ATACGACCAAGAACAG | qRT-PCR |
| | miR831TqMn_F | GTGCACGTGATCTCTCGTACTC | qRT-PCR |
| APX2 | 633/AT3G09640_R | ACTCCTGTCAGCAAACCCGAG | qRT-PCR |
| APX2 | 634/AT3G09640_F | CTTGATGATCCTCTCTTCTCCCCA | qRT-PCR |
| HSFA2 | 281/HsfA2LP | TCGTCAAGCTCAATACTTATGGATTC | qRT-PCR |
| HSFA2 | 282/HsfA2RP | CACATGACATCCCAGATCCTTGC | qRT-PCR |
| At5g38610 | 1079/At5g38610_qF | GGATAGATTGGAGGAGGCAGTGAGA | qRT-PCR |
| At5g38610 | 1080/At5g38610_qR | CACCACCGTCACCGTCGTCTCTT | qRT-PCR |
| At5g40510 | 1077/At5g40510_qF | GATTACTGGCAATTGGTATGGCTATGT | qRT-PCR |
| At5g40510 | 1078/At5g40510_qR | TTCACCTCCAGGTAGACCCATTG | qRT-PCR |
| ATHSP22.0 | 637/AT4G10250_R | TTCAGGAGATAGTTCGTGAGGTTA | qRT-PCR |
| ATHSP22.0 | 638/AT4G10250_F | ATTCTGGAGACAGTCAGCTACCT | qRT-PCR |
| HSA32 | 253/Hsa32F | CGGTCAAGCGAGTTGTGGAGGA | qRT-PCR |
| HSA32 | 254/Hsa32R | GGAATCCCGTATTCAGACATCA | qRT-PCR |
| HSP17.6A | 361/AT5G12030_q_F | GTGGACATGCCTGGAATCAA | qRT-PCR |
| HSP17.6A | 362/AT5G12030_q_R | AAACTCCCCATCCTCCTCTCC | qRT-PCR |
| HSP21 | 257/Hsp21_F | ACGTCTCTCCTTCGGATTGTTG | qRT-PCR |
| HSP21 | 258/Hsp21_R | TGTTTCTTCCTGAGACAGGCATAG | qRT-PCR |
| MIM | 555/IPS1_F | CTCGAGAACAAAAATGCCATCCCCTAGC | Franco-Zorilla et al. 2007 |
| MIM | 556/IPS1_R | GGATCCGAGGAATTCACTATAAAGAGAACATCG | Franco-Zorilla et al. 2007 |
| MIM | 557/MIM156h_I | CTTGACAGAACATAGGAGAGAGCATTCTAGAGGGA GATAA | MIM156h |
| MIM | 558/MIM156h_II | AAATGCTCTCCTATCTCTGTCAAGCTCGGTTCCC CTCG | MIM156h |
| miR156h | 465/156hoxF | ACTCGAGATAGCGAAGAGGTAAGAAAGTG | MIR156h |
| miR156h | 466/156hoxR | AGGATCCAAAACCCAATTCAAGATTGATC | MIR156h |

| | | | |
|-------------|------------------------|---------------------------------------|---------------|
| pri-miR156c | 1192/pri-miR156c_F | CTGACAGAAGAGAGTGAGCACACAAAG | qRT-PCR |
| pri-miR156c | 1193/pri-miR156c_R | CAGATAGAGCAGTGAGCACGCAAG | qRT-PCR |
| pri-miR156d | 1194/pri-miR156d_F | CAGAAGAGAGTGAGCACACAAAGGG | qRT-PCR |
| pri-miR156d | 1195/pri-miR156d_R | GTGAGCACGCAAAAGCAACCATAAC | qRT-PCR |
| SPL11 | 1028/SPL11_qF | CTGGTTGCTAGTAGCAGCCGT | qRT-PCR |
| SPL11 | 1029/SPL11_mb_s_qR | ATCCGAAGAGGTTGACAGAAGAGAG | qRT-PCR |
| SPL11 | 1030/rSPL11_mu_tmbs_qR | ATCCGAAGAGGTGCTTAACAAGCTAA | qRT-PCR |
| SPL11 | 1033/SPL11intro_n3_quF | TGGTATATGCACTTCTTCACTAGTCCT | qRT-PCR |
| SPL11 | 573/pSPL11_Nco_F | CTCCATGGCGTCTATAATTGGGTCGT | SPL11::rSPL11 |
| SPL11 | 574/pSPL11_Sac_R | GAGCTCACGATATGGGTTGATTCATAGAC | SPL11::rSPL11 |
| SPL11 | 575/SPL11cds_Sac_F | GAGCTCATGGACTGCAACATGGTATCTC | SPL11::rSPL11 |
| SPL11 | 576/SPL11cds_Xma_R | CCCGGGACAACAATTCTCACAGACCAC | SPL11::rSPL11 |
| SPL11 | 577/rSPL11_F | CGCCTTAAGCTTGTAAAGCACCTCTCGGATCCCCCT | SPL11::rSPL11 |
| SPL11 | 578/rSPL11_R | GCTTAACAAGCTTAAGGCGCGGTGGATATCTTGAGAT | SPL11::rSPL11 |
| SPL2 | 1025/SPL2_qF | GAGGTGTTAACCGCAACCTGC | qRT-PCR |
| SPL2 | 1026/SPL2_mb_s_qR | GTTGTTGAATTGACAGAAGAGAG | qRT-PCR |
| SPL2 | 1027/rSPL2_mut_mb_s_R | GTTGTTGAATTGCTTAACAAGCTAA | qRT-PCR |
| SPL2 | 1064/uSPL2_quR | TACGCATAACATAAAGCATCATCAAGA | qRT-PCR |
| SPL2 | 561/pSPL2_Nco_F | CAGTTAGGAAACCATGGATGACCGT | SPL2::rSPL2 |
| SPL2 | 562/pSPL2_Sac_R | GAGCTCAGACACTGACCACCAAAATCTG | SPL2::rSPL2 |
| SPL2 | 563/SPL2cds_Sac_F | GAGCTCATGGAGTGTAAATGCAAAGCCACC | SPL2::rSPL2 |
| SPL2 | 564/SPL2cds_Xma_R | CCCGGGCTCTGCATAACCAATCTAATCAT | SPL2::rSPL2 |
| SPL2 | 565/rSPL2_F | CGCCTTAAGCTTGTAAAGCAATTCAACACATGGGTT | SPL2::rSPL2 |
| SPL2 | 566/rSPL2_R | GCTTAACAAGCTTAAGGCGCCATCAAGATCTTGAGAA | SPL2::rSPL2 |
| SPL9 | 1023/SPL9_mb_s_R | TGATGTGGATTGACAGAAGAGAG | qRT-PCR |
| SPL9 | 1032/SPL9intron_2_quF | AGACCGATTGATACAACAGTGATGAGG | qRT-PCR |
| SPL9 | 521/SPL9_qF | AGAACATTGGATACAACAGTGATGAGG | qRT-PCR |
| TUB6 | 160/TUB6 RT R | GTCATCTGCAGTTGCGTCTT | qRT-PCR |
| TUB6 | 161/TUB6 RT F | GGTGAAGGAATGGACGAGAT | qRT-PCR |